

## AMENDMENTS

This listing of claims will replace all prior versions, and listings, of claims in the application.

### **Listing of Claims:**

Claims 1-48 (Canceled).

49. (currently amended) A method performed in a computer of simulating a metabolic capability of an *in silico* strain of a microbe, comprising:

obtaining a plurality of DNA sequences comprising most of metabolic genes in a genome of the microbe to produce an *in silico* representation of a microbe;

determining open reading frames of genes of unknown function in the microbe in said plurality of DNA sequences;

assigning a function to proteins encoded by said open reading frames by determining the homology of said open reading frames to gene sequences encoding proteins of known function in a different organism;

determining which of said open reading frames correspond to metabolic genes by determining if the assigned function of said proteins is involved in cellular metabolism;

determining substrates, products and stoichiometry of the reaction for each of the gene products of said metabolic genes;

producing a genome specific stoichiometric matrix of said microbe produced by incorporating from said substrates, products and stoichiometry into a stoichiometric matrix;

determining a metabolic demand corresponding to a biomass composition of said microbe;

calculating uptake rates of metabolites of said microbe;

combining said metabolic demands and said uptake rates with said stoichiometric matrix to produce an *in silico* representation of said microbe;

incorporating a general linear programming problem to produce an *in silico* strain of said microbe;

performing a flux balance analysis on said *in silico* strain, and

providing a visual output to a user of said analysis that simulates a metabolic capability of said strain predictive of said microbe's phenotype.

50. (previously presented) The method of claim 49, wherein said microbe is *Escherichia coli*.

51. (previously presented) The method of claim 49, wherein said genes involved in cellular metabolism comprise genes involved in central metabolism, amino acid metabolism, nucleotide metabolism, fatty acid metabolism, lipid metabolism, vitamin and cofactor biosynthesis, energy and redox generation or carbohydrate assimilation.

52. (previously presented) The method of claim 49, wherein assigning a function comprises performing a homology search using the Basic Local Alignment Search Tool (BLAST).

Claims 53-55 (canceled).

56. (previously presented) The method of claim 49, wherein said uptake rates are calculated by measuring the depletion of substrate from growth media of said microbe.

57. (currently amended) A method performed in a computer for simulating a metabolic capability of an *in silico* strain of a microbe, comprising:

- a) providing a nucleotide sequence of a metabolic gene in the microbe;
- b) determining substrates, products and stoichiometry of the reaction for the gene product of said metabolic gene, wherein said gene product having an unknown function in the microbe is assigned a function by determining homology of said nucleotide sequence to gene sequences encoding gene products of known function in a different organism;
- c) repeating steps a) and b) for most metabolic genes of said microbe to produce an *in silico* representation;
- d) producing a genome specific stoichiometric matrix produced by incorporating from said substrates, products and stoichiometry of the metabolic gene products in said microbe into a stoichiometric matrix;
- e) determining a metabolic demand corresponding to a biomass composition of said microbe;

- f) calculating uptake rates of metabolites of said microbe;
- g) combining said metabolic demands and said uptake rates with said stoichiometric matrix to produce an *in silico* representation of said microbe;
- h) incorporating a general linear programming problem to produce an *in silico* strain of said microbe;
  - i) performing a flux balance analysis on said *in silico* strain; and
  - j) providing a visual output to a user of said analysis that simulates a metabolic capability of said strain predictive of said microbe's phenotype.

58. (previously presented) The method of claim 57, wherein the microbe is *Escherichia coli*.

59. (previously presented) The method of claim 57, wherein said metabolic gene is selected from the group consisting of: genes involved in central metabolism, amino acid metabolism, nucleotide metabolism, fatty acid metabolism, lipid metabolism, vitamin and cofactor biosynthesis, energy and redox generation and carbohydrate assimilation.

60. (previously presented) The method of claim 57, wherein assigning a function comprises performing a homology search using the Basic Local Alignment Search Tool (BLAST).

Claim 61-63 (canceled).

64. (previously presented) The method of claim 57, wherein said uptake rates are calculated by measuring the depletion of substrate from growth media of said microbe.

Claim 65-67 (canceled).

68. (previously presented) The method of claim 51, wherein said genes are involved in central metabolism.

69. (previously presented) The method of claim 51, wherein said genes are involved in amino acid metabolism.

70. (previously presented) The method of claim 51, wherein said genes are involved in nucleotide metabolism.

71. (previously presented) The method of claim 51, wherein said genes are involved in fatty acid metabolism.

72. (previously presented) The method of claim 51, wherein said genes are involved in lipid metabolism.

73. (previously presented) The method of claim 51, wherein said genes are involved in vitamin and cofactor biosynthesis.

74. (previously presented) The method of claim 51, wherein said genes are involved in energy and redox generation.

75. (previously presented) The method of claim 51, wherein said genes are involved in carbohydrate assimilation.

76. (previously presented) The method of claim 59, wherein said genes are involved in central metabolism.

77. (previously presented) The method of claim 59, wherein said genes are involved in amino acid metabolism.

78. (previously presented) The method of claim 59, wherein said genes are involved in nucleotide metabolism.

79. (previously presented) The method of claim 59, wherein said genes are involved in fatty acid metabolism.

80. (previously presented) The method of claim 59, wherein said genes are involved in lipid metabolism.

81. (previously presented) The method of claim 59, wherein said genes are involved in vitamin and cofactor biosynthesis.

82. (previously presented) The method of claim 59, wherein said genes are involved in energy and redox generation.

83. (previously presented) The method of claim 59, wherein said genes are involved in carbohydrate assimilation.